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Antifungal property of *Ficus septica* leaf ethanolic extract

Catherine Fugaban-Hizon
Cagayan State University, Andrews Campus, Tuguegarao, Philippines

Abstract
The study aims to determine the antifungal property of *Ficus septica* leaf ethanolic extract against Candida albicans and Aspergillus Niger. The antimicrobial susceptibility tests were conducted with four different level of concentrations at 100%, 75%, 50% and 25% respectively of *Ficus septica* leaf ethanolic extract and Itraconazole was used as positive control. The zone of clearing was carefully noted using caliper. Results reveal the zones of clearing against Candida albicans at 50%, 75% and 100% concentration of *Ficus septica* leaf ethanolic extract were 31.3 mm, 33 mm, and 32.2 mm, respectively. As compared to Itraconazole, the results revealed that it has inhibitory activity with sensitive reactivity against Candida albicans and only intermediate activity against Aspergillus Niger at 50% concentration.
The study revealed that the leaf ethanolic extract of *Ficus septica* exhibited an antifungal activity against Candida albicans and Aspergillus Niger.

Keywords: antifungal property, *Ficus septica*, aspergillus Niger, candida albicans

Introduction
In recognition of the rapidly-emerging global public health threats of antimicrobial resistance, the World Health Organization (WHO) has urged governments throughout the world to take an urgent concerted action to address the consequences of Anti-microbial Resistance (AMR) since 2011. In response to this call, the Office of the President of the Philippines signed the Administrative Order (AO) no. 42 series of 2014 entitled "Creating an Inter-Agency Committee for the Formulation and Implementation of a National Plan to Combat Antimicrobial Resistance in the Philippines" to bring together all key partners across to mitigate and control AMR. The Philippines have also been conducting extensive research in the traditional medicines used by Filipinos in earlier times. However, thousands of plant species are still not discovered and studied extensively. Thus, Philippine Institute of Traditional and Alternative Health Care (PITAHC) were created under Republic Act (RA 8423) otherwise known as the Traditional and Alternative Medicine Act (TAMA) of 1997. Traditional medicine has become significant in our health care system and has been passed from generation to generation. *Ficus septica* is one of the oldest traditional medicine used by the Filipinos. As stated by Tsai in his study on 2006, the leaves were used as cure for skin disease, appendicitis, abscesses, poisonous snakebites and shortness of breath. According to Stuart in 2015, folks believed that it can cure skin diseases, gastrointestinal problems and others.
Many phytochemical studies of the *Ficus septica* has been conducted and showed to have anti-microbial properties and anti-oxidant properties. Presence of coumarins, flavonoids, saponins, steroids, alkaloids and tannins has been reported in the extracts of *Ficus septica*. In 2005, Berg et al., include (-)-tilosrebin (hauptalkaloid), tiloforin, septisin, and antofin, but it also contains flavonoids. A study of Kuo et al., in 2002 has reported the isolation of seven triterpene derivative, 13, 27-cycloursan-3β-yl acetate, and two lignans from the non-alkaloidal fractions of the stem of *Ficus septica*.
Alkaloids rank among the most efficient and therapeutically significant plant substances; hence they are widely used in medicine for the development of drugs (Harborne, 1998). They are generally toxic to micro-organisms (Mattei et al. 2014) [11] by inhibiting spore germination and mycelial growth significantly substantiating their fungicidal potentials (Iwu et al. 1999) [9]. Flavonoids also act by inhibiting DNA and RNA synthesis, energy metabolism, and cytoplasmic membrane function of microorganism (Galpa, 2013) [6] and tannins have been reported to hinder the tertiary structure of proteins by directly binding with the proteins thus effectively inhibit the function of ABC transporters that enables the fungal pathogens resistant to the administered drug (Ansari et al. 2013) [1]. Thus, these plant extracts can possibly be used to produce alternative forms of antimicrobials (Vital, Velasco, Demigillo & Rivera, 2010) [20].
In the European Journal of Clinical Microbiology and Infectious Diseases in 2017, Aspergillosis and Candidiasis has the most cases of fungal infections in the Philippines. Although, Candida species has the most common anti-fungal resistance, other types of less common fungi such as Aspergillus niger is also a problem. Aspergillus infections are now developing resistance to the first-line treatment, thus, threaten the effectiveness of the life-saving medications. The Center of Disease and Control and Prevention (CDC) reported that Aspergillus is the leading cause of invasive mold infections, with an estimated 300,000 cases worldwide every year. Depending on

location, up to 12% of Aspergillus infections are estimated to be resistant to antifungal medications. Nigam et al., in 2017 reported the increased use and over the counter sale of antifungal agents in recent years has also resulted in the development of resistance to these drugs. Fungi are growing increasingly resistant to drugs. In 2003, cases of terbinafine resistance and griseofulvin was reported.

With the increasing cases of diseases and anti-microbial resistance, this study was conducted to determine the antifungal activity of *Ficus septica* leaf ethanolic extract that is economical, energy efficient, cost effective and help reduce cases of anti-microbial resistance.

Materials and Methods

Collection of Bacterial and Fungal Samples

The inhibitory effect of *Ficus septica* leaf ethanolic extract was used against Aspergillus niger (USTCMS 1323) and Candida albicans (USTCMS 1235). The samples were collected from the University of Santo Tomas, Research Center for the Natural and Applied Sciences (UST-RCNAS) Microbiology Laboratory.

Plant collection and Extract Preparation

Fresh *Ficus septica* leaves were collected in Rizal, Cagayan. Leaves were thoroughly washed with distilled water for three times. The leaves were air dried under shade. The dried plant was pulverized using a disintegrator. The powdered samples were kept in airtight containers for extraction purposes. 150g of powdered *Ficus septica* leaves was mixed in 1,350ml of ethanol in erlenmeyer flask, covered with aluminum foil and stored for five days.

The mixture was filtered using analytical filter paper and the resulting filtrate placed in rotary evaporator. After the procedure, the extract was collected and stored in the refrigerator.

Antimicrobial Susceptibility Testing (Kirby-Bauer Disk Diffusion Method)

Media Preparation.

Thirty-eight grams (38g) of Potato Dextrose Agar was suspended in 1L distilled water and the mixed solution was placed on a hot plate until the agar has completely dissolved. Using autoclave, the media was sterilized at 121°C for 15-20 minutes. Approximately 15mL of melted Mueller Hinton agar was poured into dry and sterile Petri dish and allowed the medium solidify.

Preparation of the fungal inoculum.

A loopful of 16-24-hour old of Candida albicans was inoculated into 9 mL sterile 0.85% NaCl solution. The inoculum was compared with 0.5 McFarland Standard to obtain a standardized inoculum equivalent to $1.5x10^8$ cells for the conduct of assays (Reller et al., 2013) [15].

One agar disc (6 mm) from the marginal colony of a 7-day-old culture of Aspergillus niger was inoculated onto 20 mL sterile 0.85% NaCl solution with 100 µL Tween-20 (Tian et al. 2013) [19]. The fungal suspension was gently vortexed and allowed to stand for 15 minutes for the sedimentation of the hyphal fragments. The upper supernatant containing conidia was then carefully transferred to a new sterile tube.

Streaking of microorganisms

A sterile cotton swab was moistened into the test organism (inoculum) suspension. Aseptically, the test organism was swabbed onto a solidified Mueller Hinton agar plate by streaking the swab over the entire surface of the agar plate three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum on the surface of the medium. Finally, it was allowed to pass the swab round edge of the agar surface and left the inoculum to dry for a few minutes at room temperature with lids closed.

Paper disc diffusion method

The tests were conducted with four different level of concentrations at 100%, 75%, 50% and 25% respectively of *Ficus septica* leaf ethanolic extract. The disc was treated with respective treatments (25 µl) using micro pipette and allowed to dry for few minutes. Using a pair of sterile forceps, the discs was placed on the inoculated Mueller Hinton agar plate. Each disc was gently pressed down to ensure even contact with the medium. Fungal plates were incubated at 37°C for 4-7 days. The activity of each extract was tested in triplicate. Itraconazole discs served as a positive control discs were subjected on the agar plates. Measurement of clearing for fungi were noted after performing the procedure using caliper.

Results and Discussions

Table 1 presents the summary of the mean zone of clearing of *Ficus septica* leaf ethanolic extract in millimeter (mm). At 50%, 75% and 100% concentration of *Ficus septica* ethanolic extract, the recorded zone of clearing against Candida albicans were 31.3 mm, 33 mm and 32.2 mm, respectively. On the other hand, at 50%, 75% and 100% concentration, the zone of clearing showed 16.3 mm, 11mm and 23.5mm respectively against Aspergillus Niger. As compared to a positive control (Itraconzole), the data implies that it exhibited a complete inhibitory activity with sensitive reactivity against Candida albicans and only intermediate at 50% against Aspergillus Niger. The antifungal activity of *Ficus septica* revealed in this study was documented also in the study conducted by Vital et al. (2010) where antifungal assay for *Ficus septica* extract showed that it inhibited Candida

albicans. This can be attributed to the fact that *Ficus septica* contain two indolizidine antimicrobial alkaloid namely ficuseptine, 4,6-bis-(4-methoxyphenyl)- 1,2,3-trihydroindolizidinium chloride, and antofine (Damu et al., 2005; Baumgartner, Erdelmeier, Wright, Rali & Sticher, 1990 as cited by Ragasa et al., 2016) [2, 14, 15].
In the study of Fugaban-Hizon in 2021, FTIR analysis shows that there are 4 peaks of the *Ficus septica* ethanolic extract. This can prove the presence of an amino or methyl ethers from flavonoids or alkaloids in *Ficus septica*. Bortolus et.al (2019) [4] isolated a compound containing a methoxy group which has a potential antifungal activity.

Table 1: Antifungal activity of *Ficus septica* leaf ethanolic extract at various concentrations

Treatments	$\bar{x}$ Zone of Clearing (mm)	
	Fungi	
	C. albicans	A. niger
F. septica ethanolic extract		
25%	6	6
50%	31.3	16.3
75%	33	11
100%	32.2	10.6
Itraconazole (Positive control for fungi)	35.7	23.5
*CLSI Standard for zone of inhibition	Itraconazole less than 13 mm – resistant 14-22 mm- intermediate 23 mm or more - sensitive	

Conclusion
The study revealed that *Ficus septica* leaf ethanolic extract exhibited an antifungal activity against Candida albicans and Aspergillus Niger. The presence of a compound containing a methoxy group has a potential antifungal activity.

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University of Santo Tomas, Research Center for the Natural and Applied Sciences (RCNAS), 2/F Thomas Aquinas Research Complex, Espana Manila, 1015 Philippines Cagayan State University, Andrews Campus, Tuguegarao City Department of Science and Technology Region 02

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